NUCLEIC ACID SEQUENCES FROM DIABROTICA VIRGIFERA VIRGIFERA LECONTE AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention is in the field of molecular biology; more particularly, the present invention relates to nucleic acid sequences from the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. The invention encompasses nucleic acid molecules that encode proteins and fragments of proteins. In addition, proteins and fragments of proteins so encoded and antibodies capable of binding the proteins are encompassed by the present invention. The invention also relates to methods of using the disclosed nucleic acid molecules, proteins, fragments of proteins, and antibodies, for example, for gene identification and analysis, and preparation of constructs.

BACKGROUND OF THE INVENTION

[0002] The western corn rootworm (WCRW), *Diabrotica virgifera virgifera* LeConte, is a major pest of corn in the United States. The western corn rootworm overwinters in the egg stage in fields where corn was grown the previous season. The eggs hatch from late May through June. Thereafter, the larvae pass through 3 larval stages, or instars, feeding upon the corn root system. Following completion of larval development, the larvae transform into pupae, which are white and immobile. Adults of western corn rootworms begin to emerge in early July and continue to emerge from the pupae stage into August. Adult beetles feed on the corn foliage and silk. Female beetles lay the vast majority of their eggs in the soil of cornfields during August and early September. Western corn rootworm larvae can survive only on corn and a few other species of Poaceae (Branson and Ortman, *J. Econ. Entomol.* 60: 201-203 (1967); Branson and Ortman, *J. Econ. Entomol.* 60: 201-203 (1967)). Larval root feeding decreases plant vigor by reducing the water and nutrients supplied to the developing corn plants. Extensive root damage weakens the root system and makes the plants more susceptible to lodging (plants lean over or elbow), which eventually reduces corn yield and often results in death of the plant. Lodged plants are difficult to harvest resulting in further yield losses. The western corn rootworm adults feed upon corn leaves, which can slow plant growth and, on rare occasions, kill plants of some corn varieties. The western corn rootworm cause economic losses throughout the Midwest and in certain eastern and northeastern states where corn is produced.

[0003] Control of corn rootworms has been partially addressed by crop rotation. However, economic demands on the utilization of farmland restrict the use of crop rotation. In addition, the spread of at least one strain of rootworm has been documented in which female oviposition occurs in soybean fields, which further complicates crop rotation strategies. Therefore, chemical insecticides are relied upon most heavily to guarantee the desired level of control. Over $250 million worth of insecticides are applied annually to control corn rootworms alone in the United States. Even with insecticide use, rootworms still can cause over $750 million worth of crop damage each year. The use of chemical insecticides to control corn rootworm has several drawbacks. Continual use of insecticides has allowed resistant insects to evolve. Situations such as extremely high populations of larvae, heavy rains, and improper calibration of insecticide application equipment can result in poor control. Chemical insecticides used for corn rootworm control often raises environmental concerns such as contamination of soil and of both surface and underground water supplies, because many of them are toxic to humans, wildlife and other nontarget species. As a result, much research has been concentrated in the area of biopesticides.

[0004] The advantage of using biopesticides is that they are generally less harmful to non-target organisms and the environment as a whole compared to chemical pesticides. The most widely used biopesticide is *Bacillus thuringiensis* (Bt), which is a spore-forming gram-positive bacterium. During sporulation, *B. thuringiensis* produces proteinaceous inclusions which are composed of proteins known as insecticidal crystal proteins (ICPs), Cry proteins, or delta-endotoxins. These proteins are toxic to a variety of insect species including orders Lepidoptera, Coleoptera, Diptera, Hemoptera, Hymenoptera, Orthoptera, and Mallophaga (Beegle and Yamamoto, *Can. Entomol.* 124:587-616; Feitelson, Advanced Engineered Pesticides (L. Kim, ed.), Marcel Dekker, Inc., New York (1993), pp. 63-71; Feitelson, et al., *Bio/Technology* 10:271-275; U.S. Pat. No. 4,948,734 (1990)). Due to their high specificity for particular insect pests and their safety for man and the environment, ICPs have been used as biopesticides for the last three decades.

[0005] It has been established that the Bt toxins function in the brush border of the insect midgut epithelial cells as described by Gill et al., *Annu. Rev. Entomol.* 37: 615 (1992). Specific binding of Bt toxins to midgut brush border membrane vesicles has been reported by Hofmann et al., *Proc. Natl. Acad. Sci. USA* 85: 7844 (1988); Van Rie et al., *Eur. J. Biochem.* 186: 239 (1989); and Van Rie, *J. et al. Appl. Environ. Microbiol.* 56: 1378 (1990). It is believed that the specificity of Bt toxins is determined by their specific interaction with receptors in insects' guts. It is advantageous to identify and/or isolate receptors as targets for insecticidal peptides in the guts of western corn rootworms. It is further advantageous to develop target-based screens to produce insecticidal peptides.

[0006] A cDNA (or complementary DNA) library, which is constructed from mRNA purified from WCRW intestine, can be one valuable source for isolating receptor protein genes. Construction of cDNA libraries is well-known in the art and a number of cloning strategies exist Random clones from a cDNA library can be sequenced from both 3' and 5' ends to generate expressed sequence tags (ESTs), which can represent copies of up to the full length transcript (McCombie, et al., *Nature Genetics,* 1:124-130 (1992); Kurata, et al., *Nature Genetics,* 8: 365-372 (1994); Okubo, et al., *Nature Genetics,* 2: 173-179 (1992)). Typically, only single run sequence data is obtained from the cDNA library (Adams, et al., *Science* 252:1651-1656 (1991)). Automated single run sequencing typically results in an approximately 2-3% error or base ambiguity rate. (Boguski, et al., *Nature Genetics,* 4:332-333 (1993)). Between 150-450 nucleotides of sequence information is usually generated as this is the length of sequence information that is routinely and reliably produced using single run sequence data.

[0007] ESTs have been found to be useful for similarity searches and mapping (Adams, et al., *Science* 252:1651-1656 (1991)). Sequence comparisons and similarity analysis would allow the identification of receptors for insecticidal peptides and then full-length cDNA constructs can be obtained using several methods (Land, et al., *Nucleic Acids*